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Changes in natural killer cell phenotype in patients with post-viral fatigue syndrome

L. J. A. MORRISON, W. H. M. BEHAN & P. O. BEHAN *Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland*

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SUMMARY

We analysed peripheral blood $CD56^+$ natural killer (NK) cell subsets in 23 carefully characterized patients with post-viral fatigue syndrome (PFS), compared with 19 healthy controls, using fluorochrome-conjugated, specific monoclonal antibodies and the FACScan. We found significantly increased percentages of $CD56^+$, and especially $CD56^{bright+}$ NK cells in PFS patients. We also found significantly increased percentages of $CD56^+$ high affinity interleukin-2 (IL-2) receptor $(CD25)^+$ and $CD56^+$ transferrin receptor $(CD71^+)$ subsets of cells, most of which also stained brightly for CD56. Also, we found an increased percentage of $CD56^+$ $CD3^+$ cells, many of which stained brightly for CD56, although there was no increase in the percentage of $CD56^-$ $CD3^+$ T cells in these patients. These observations, in conjunction with very low percentage of $CD56^-$ $CD25^+$ cells, suggest that there is a preferential involvement of this minor subset of $CD56^+$ $CD3^+$ T cells in PFS. Finally, a decreased percentage of $CD56^+$ Fc gamma receptor $(CD16)^+$ NK cells was identified, which suggests a reduced capacity for antibody-dependent cellular cytotoxicity in PFS patients. Subsets of $CD56^+$ NK cells co-expressing CD2, CD4 or CD8 did not show any significant difference between PFS patients and healthy controls. These phenotypic changes provide laboratory evidence of immunological abnormalities in this syndrome, and, we suggest, may be consistent with persistent viral infection.

Keywords natural killer cells phenotype post-viral fatigue syndrome

INTRODUCTION

The post-viral fatigue syndrome (PFS) is a disorder of acute onset with a prolonged course, developing after a viral infection, and characterized by severe fatigue, myalgia and a variety of psychiatric symptoms. One-third of patients also show evidence of cardiac involvement (Behan & Behan, 1988). Routine laboratory tests are normal but specialized investigations, i.e. electromyography and nuclear magnetic resonance testing provide evidence of muscle damage (Jamal & Hansen, 1989; Arnold *et al.*, 1984). Several types of viruses have been implicated in its aetiology including enteroviruses, especially coxsackie, Epstein–Barr, hepatitis B and varicella (Behan, Behan & Bell, 1985; Straus *et al.*, 1985; Yousef *et al.*, 1988). Enteroviral nucleic acid has now been identified in up to 53% of muscle biopsies from these cases (Archard *et al.*, 1988), suggesting that a persistent viral infection may be causing the functional abnormalities.

Evidence of a specific immune response to coxsackie virus in PFS is conflicting and difficult to interpret (Behan *et al.*, 1985; Miller *et al.*, 1990). We decided to examine the natural killer (NK) lymphocyte subset, because these cells, defined functionally by their ability to lyse certain target cells without prior sensitization or MHC restriction, have been shown to play a significant role in the immune response to a wide range of viral infections in animals (Welsh, 1981). During acute viral infections they respond rapidly to viral challenge, mounting both a proliferative and cytolytic response several days before a specific T cell response can be mobilized. In persistent viral infection in animals, NK activity remained increased for several months (Bukowski, Biron & Welsh, 1983). With regard to humans, similar enhancement of NK activity has been identified in acute viral infections (Perrin, Tishon & Oldstone, 1977; Ennis *et al.*, 1981). Little was known of their role in chronic viral infections until recently, when Caligiuri *et al.* (1987) reported abnormalities in NK cell phenotype in patients with chronic Epstein–Barr virus infection. We decided therefore to carry out a detailed phenotypic analysis of NK cells in our group of carefully characterized patients with PFS.

Correspondence: L. J. A. Morrison, Institute of Neurological Sciences, Southern General Hospital, 1345 Govan Road, Glasgow G51 4TF, UK.

SUBJECTS AND METHODS

Patients and controls

Twenty-three patients with PFS were included in the study (10 men, aged 16–50 years, mean 31, and 13 women, aged 18–48

years, mean 30). They were selected because they all had severe fatigue that had been present for more than 1 year and because they had already been extensively investigated to exclude the many other conditions which enter into the differential diagnosis of this syndrome.

Their symptoms were overwhelming fatigue made worse by exercise, myalgia, and depression, with poor concentration and short-term memory. Additional symptoms included disturbance of the sleep pattern, palpitations and pains in the chest, dizziness and excessive sweating. A febrile, viral-type illness, with upper respiratory or gastrointestinal symptoms, of such severity that the patient was confined to bed for several days, was the precipitating factor in all cases. Before their illness, all 23 had considered themselves in good physical health.

Nineteen normal healthy control subjects with no history of recent or chronic viral infections, all of whom denied symptoms of PFS as described above, were also included in the study (10 men, aged 21–50, mean 30, and nine women aged 22–48 years, mean 28).

Preparation of peripheral blood mononuclear leucocytes for immunofluorescence

Samples of peripheral venous blood, drawn from patients with PFS and healthy control subjects, were anti-coagulated with 3·8% sodium citrate, and the mononuclear leucocytes separated by density gradient centrifugation using lymphocyte separation medium (Flow Labs., Irvine, UK). The mononuclear cell layer was aspirated and washed twice in RPMI 1640 containing bicarbonate, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 10^{-5} M mercaptoethanol and 10% fetal calf serum (FCS) (heat-inactivated at 56°C for 30 min). The mononuclear cells were then resuspended in cold RPMI 1640 containing only bicarbonate, 0·2% NaN_3 and 1% FCS at a concentration of $3–5 \times 10^6$/ml.

Phenotypic analysis of NK cells by two-colour immunofluorescence

Two-colour immunofluorescence analysis was accomplished by incubating mononuclear cells for 40 min at 4°C in darkness with PE-conjugated monoclonal antibody (MoAb) to Leu-19 (CD56 or NKH-1), which was used as a *pan*-NK marker, with one of the following group of FITC-conjugated MoAbs specific for various T cell antigens and markers of immunological activation and cell division: anti-Leu2a (CD8), anti-Leu4 (CD3), anti-Leu3a (CD4), anti-Leu5b (CD2), anti-Leu11a (CD16), anti-high-affinity interleukin-2 (IL-2) receptor (CD25), and anti-transferrin receptor (CD71). The latter is a marker for both immunological activation (Larrick & Cresswell, 1979) and cell division (Hamilton, Wada & Sussman, 1979; Shindelman, Ortmeyer & Sussman, 1981). Background fluorescence was determined by incubating cells with PE-conjugated or FITC-conjugated non-reactive MoAbs of a similar immunoglobulin class. All fluorochrome-conjugated MoAbs were purchased from Becton Dickinson, Oxford, UK.

Following incubation with conjugated MoAbs, the mononuclear cells were washed twice with cold RPMI 1640 containing 0·2% NaN_3 and 1% FCS, then fixed with 1% paraformaldehyde plus 0·2% NaN_3 in phosphate-buffered saline. Stained and fixed cells were usually stored at 4°C in darkness for 24–48 h before analysis.

The simultaneous analysis of green and red fluorescence was obtained from a single laser exciting both FITC and PE at 488 nm using a FACScan (Becton Dickinson). Electronic compensation for the small overlaps of green and red fluorescence yielded signals essentially identical to those with either reagent alone. In total, 10 000 cells were analysed in each sample and the results were displayed as a 10% linear contour plot expressing cell density, log green (abscissa) *versus* log red (ordinate) fluorescence.

All experiments were performed in a single blind fashion. Identification of the samples was not revealed until all data were permanently recorded.

Statistical analysis

Statistical analysis was carried out using the Kolmogorov–Smirnov two-sample test of significance for non-parametrically distributed data.

RESULTS

$CD56^+$ mononuclear cells

The mononuclear cells from PFS patients and healthy controls were stained with PE-anti-CD56 alone, and the number of $CD56^+$ cells expressed as a percentage of the total number of mononuclear cells for each individual. Patients with PFS showed a significantly greater ($P < 0·05$) percentage of $CD56^+$ mononuclear cells (mean 26·45%, s.e.m. 3·33%) than did normal control donors (18·13 ± 1·41%).

$CD56^{bright+}$ mononuclear cells

Patients with PFS also showed a very significantly greater percentage ($P < 0·0001$) of mononuclear cells which stained brightly for CD56 ($CD56^{bright+}$) (7·56 ± 2·72%) than did healthy control donors (0·4 ± 0·03%).

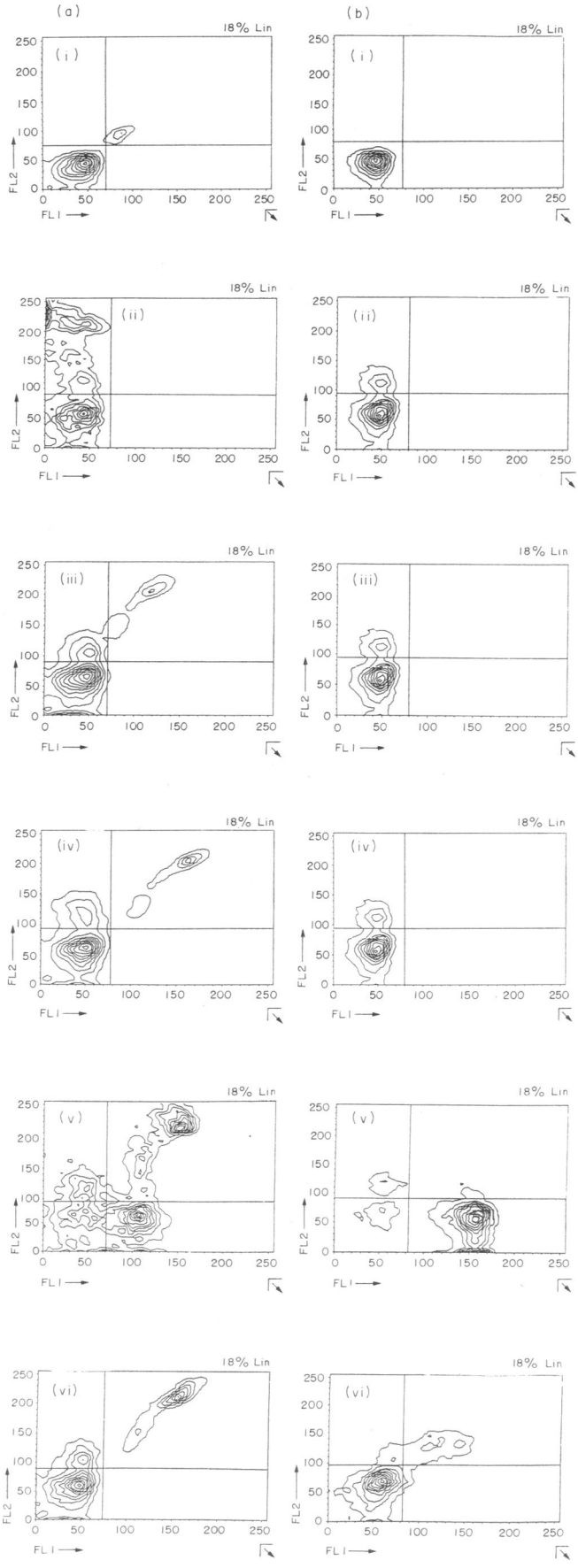
When the number of $CD56^{bright+}$ mononuclear cells was expressed as a percentage of the total $CD56^+$ mononuclear cells for each individual, the difference between PFS patients (20·99 ± 3·86%) and healthy controls (2·45 ± 0·07%) was also highly significant ($P < 0·00001$).

Fig. 1a(ii) shows mononuclear cells derived from a typical patient with PFS, and Fig. 1b (ii) mononuclear cells derived from a typical normal control donor, both of which were stained with PE-anti-CD56. Fig. 1a(ii) shows the large proportion of mononuclear cells staining brightly for CD56 in the PFS patient, compared with the virtual absence of such cells in the normal control (Fig. 1b(ii)).

The number of mononuclear cells co-expressing CD56 and one of several T cell antigens (CD2, CD3, CD4, CD8), the Fc-γ receptor antigen CD16, the high affinity IL-2 receptor antigen CD25, or the transferrin receptor antigen CD71, was expressed as a percentage of the total number of $CD56^+$ mononuclear cells for each PFS patient and normal control. In this way subsets of $CD56^+$ cells were defined.

$CD56^+CD25^+$ NK cells

As shown in Fig. 2, patients with PFS showed a significantly higher percentage of $CD56^+CD25^+$ NK cells ($P < 0·0001$) (25·07 ± 5·56%) than did normal control donors (4·66 ± 2·35%). PFS patients (0·86% ± 0·18%) and normal control donors (0·84 ± 0·12%) had a similarly low percentage of $CD56^-CD25^+$ mononuclear cells (not shown). The majority of $CD56^+CD25^+$



cells from PFS patients stained brightly for CD56, a typical example of which is shown in Fig. 1a(iii). These cells were seldom seen in normal controls (Fig. 1b(iii)).

$CD56^+CD71^+$ NK cells

As shown in Fig. 3, PFS patients showed a significantly higher percentage of $CD56^+CD71^+$ NK cells ($P<0{\cdot}00001$) ($20{\cdot}86\pm4{\cdot}44\%$) than did healthy control donors ($3{\cdot}45\pm0{\cdot}73\%$). PFS patients ($0{\cdot}98\pm0{\cdot}62\%$) and healthy control donors ($0{\cdot}52\pm0{\cdot}1\%$) had a similarly low percentage of $CD56^-CD71^+$ mononuclear cells (not shown). The majority of $CD56^+CD71^+$ cells from PFS patients stained brightly for CD56, a typical example of which is shown in Fig. 1a(iv). These cells were seldom seen in normal controls (Fig. 1b(iv)).

$CD56^+CD3^+$ mononuclear cells

As shown in Fig. 4, patients with PFS showed a significantly higher percentage of $CD56^+CD3^+$ mononuclear cells ($P<0{\cdot}01$) ($42{\cdot}47\pm4{\cdot}05\%$) than did healthy control donors ($21{\cdot}82\pm2{\cdot}92\%$). The percentage of $CD56^-CD3^+$ cells did not differ significantly between PFS patients ($58{\cdot}96\pm3{\cdot}04\%$) and healthy control donors ($61{\cdot}87\pm2{\cdot}95\%$) (not shown). The majority of $CD56^+CD3^+$ cells from PFS patients also stained brightly for CD56, a typical example of which is shown in Fig. 1a(v). These $CD56^{bright+}CD3^+$ cells were seldom seen in normal control donors (Fig. 1b(v)).

$CD56^+CD16^+$ NK cells

As shown in Fig. 5, patients with PFS showed a significantly lower percentage of $CD56^+CD16^+$ NK cells ($P<0{\cdot}001$) ($54{\cdot}16\pm6{\cdot}31\%$) than did normal control donors ($86{\cdot}54\pm2{\cdot}67\%$). The percentages of $CD56^+CD16^-$ mononuclear cells did not differ significantly between PFS patients and normal control donors. A large proportion of $CD56^+CD16^+$ cells from PFS patients stained brightly for CD56, a typical example of which is shown in Fig. 1a(vi). These cells were seldom seen in normal controls (Fig. 1b(vi)).

$CD56^+CD8^+$, $CD56^+CD4^+$, and $CD56^+CD2^+$ NK cells

Results showed that the percentages of mononuclear cells co-expressing CD56 and any one of the T cell antigens CD8, CD4

Fig. 1. Two-colour immunofluorescence analysis of peripheral blood mononuclear cells from a typical PFS patient is shown in a(i)–(vi) and for a typical normal control in b(i)–(vi). Background fluorescence was determined by incubating mononuclear cells with a non-reactive PE-conjugated MoAb and a non-reactive FITC-conjugated MoAb (a(i) and b(i)). Markers defining quadrants were positioned to include >98% of unstained cells in the lower left quadrant. Results are presented as a contour plot of cell density (log red fluorescence (PE) (FL2) on the ordinate *versus* log green fluorescence (FITC) (FL1) on the abscissa) for each combination. In subsequent panels, cells positive for red fluorescence alone appear in the upper left quadrant, cells positive for green fluorescence alone appear in the lower right quadrant, and cells positive for both red and green fluorescence appear in the upper right quadrant. Mononuclear cells were stained with PE-anti CD56 alone (panels (ii)); PE-anti CD56 and FITC-anti high-affinity Il-2 receptor (CD25) (panels (iii)); PE-anti CD56 and FITC-anti human transferrin receptor (CD71) (panels (iv)), PE-anti CD56 and FITC-anti CD3 (panels (v)); and PE-anti CD56 and FITC-anti Fc gamma receptor antigen (CD16) (panels (vi)). Correlated measurements of fluorescence are presented as 10% linear contour plots.

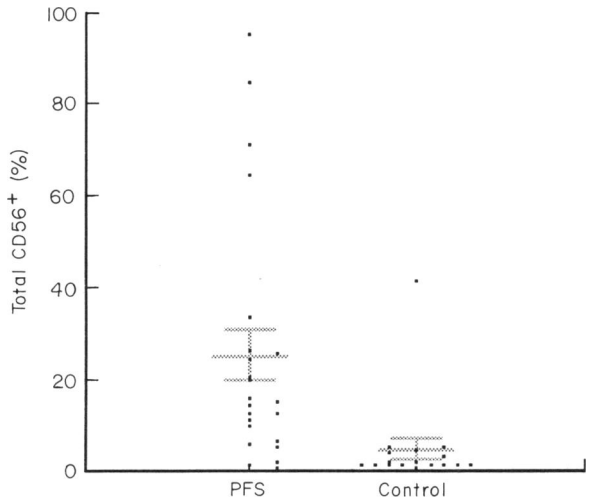


Fig. 2. Percentage of total CD56 positive mononuclear cells co-expressing the high affinity IL-2 receptor antigen CD25 (mean ± s.e.m.).

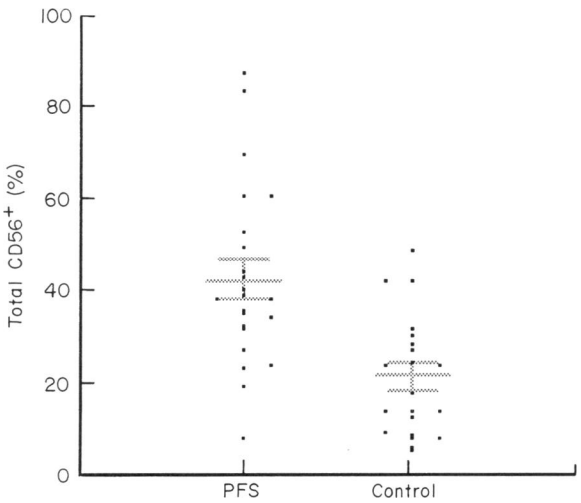


Fig. 4. Percentage of total CD56 positive mononuclear cells co-expressing CD3 (mean ± s.e.m.).

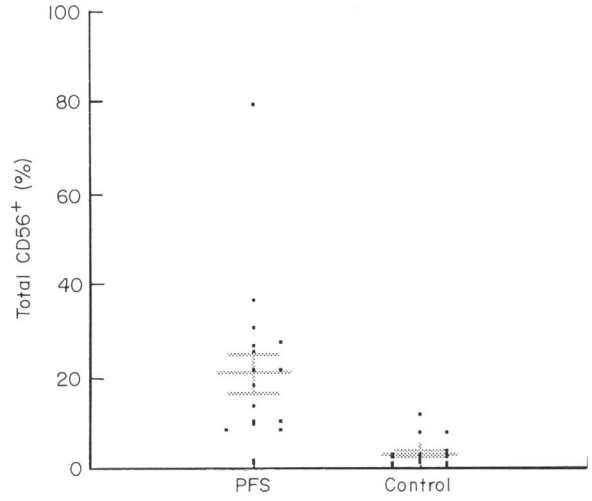


Fig. 3. Percentage of total CD56 positive mononuclear cells co-expressing the transferrin receptor CD71 (mean ± s.e.m.).

or CD2, did not differ significantly between PFS patients and healthy controls.

DISCUSSION

We have shown several changes in phenotypic expression of NK cells in patients with PFS. As little is known of the function of the various phenotypically defined subsets of NK cells, changes in function resulting from altered phenotypic expression must remain the object of speculation.

We used the *pan*-NK marker anti-CD56 to identify NK cells. This monoclonal antibody reacts with NKH-1 antigen (Lanier *et al.*, 1986), a 220-kD glycoprotein known to be present on at least 95% of NK cells, and on approximately 15% of normal peripheral blood mononuclear cells (Griffin *et al.*, 1983; Hercend *et al.*, 1985; Lanier *et al.*, 1986). It is not completely specific for NK cells, since it is also present on a small subpopulation of $CD3^+$ T lymphocytes comprising less than 5% of all peripheral blood lymphocytes (Lanier *et al.*, 1986). Using this *pan*-NK marker we have found significantly increased percentages of $CD56^+$, and especially $CD56^{bright+}$ NK cells in patients with PFS compared with healthy controls.

Lanier *et al.*, (1986) was the first to report that a small number of NK cells can be distinguished from the majority because of the high density expression of CD56 (NKH-1) antigen. These $CD56^{bright+}$ cells were shown to comprise less than 2% of peripheral blood mononuclear cells, or less than 5% of the entire NK cell population in normal individuals. Caligiuri *et al.* (1990) have confirmed these findings, and also reported that these cells were unique among normal mononuclear cells in that they expressed constitutively, functional, high-affinity IL-2 receptors (CD25), and had the ability to proliferate rapidly in response to minimal concentrations of serum IL-2, but were able to do so in the absence of any antigenic stimulus.

At present, little is known of the role played by $CD56^{bright+}$ NK cells in relation to human disease. Caligiuri *et al.* (1990) have suggested that the expression of functional high-affinity IL-2 receptors (CD25) by $CD56^{bright+}$ cells would allow this minor NK cell subset to respond rapidly and non-specifically to virus or virus-infected cells before a specific T cell response was generated. Our results have shown that patients with PFS had a significantly increased percentage of both $CD56^+CD25^+$ (Fig. 2) and $CD56^+CD71^+$ (Fig. 3) subsets of NK cells compared with normal controls. Fig. 1a(iii) and (iv) shows that the majority of these subsets of NK cells also stained brightly for CD56. These findings would seem to indicate that this significantly expanded population of high-affinity IL-2 receptor $(CD25)^+$ NK cells, including the $CD56^{bright+}$ population, plays a significant role in the immunopathology of PFS.

Interestingly, we also found that the percentages of $CD56^-CD25^+$ and $CD56^-CD71^+$ mononuclear cells were very low in PFS patients, indicating the absence of actively proliferating $CD56^-$ T cells in this syndrome. The presence of a significantly increased percentage of high-affinity IL-2 receptor $(CD25)^+$ NK cells, in the absence of a significantly increased percentage of $CD56^-$ high-affinity IL-2 receptor $(CD25)^+$

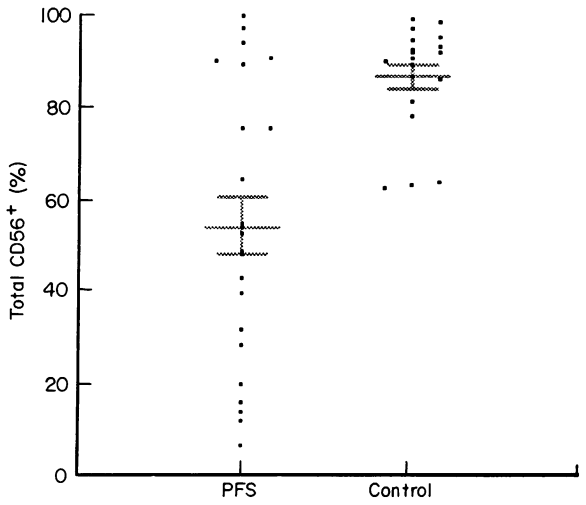


Fig. 5. Percentage of total CD56 positive mononuclear cells co-expressing the Fcγ receptor antigen CD16 (mean ± s.e.m.).

T cells is unusual, and may indicate an abnormal immunological response in these patients.

The percentage of $CD56^+CD3^+$ mononuclear cells was also found to be significantly greater in PFS patients than in normal controls. The phenotypic marker CD3 present on these NK cells is also present on most $CD56^-$ T cells, but the percentage of $CD56^-CD3^+$ T cells was not significantly increased in PFS patients relative to normal controls. These data indicate the selective expansion of $CD56^+CD3^+$ cells, which normally constitute only 20–25% of the total $CD56^+$ population, and less than 5% of all peripheral blood lymphocytes (Lanier *et al.*, 1986). We also found that the majority of these cells stained brightly for CD56 (Fig. 1a(v)). As the functional role of this subpopulation of cells is not yet known, the increased percentage we detected—including cells which stained brightly for CD56—is of unknown significance in this syndrome.

As shown in Fig. 5, the percentage of $CD56^+CD16^+$ NK cells in patients with PFS was significantly reduced compared to normal controls. Fig. 1a(vi) shows that a proportion of these NK cells also stained brightly for CD56. Several studies have documented the expression of CD16 by 80–90% of NK cells in normal peripheral blood (Lanier *et al.*, 1983; Perussia *et al.*, 1984). The reduced percentage of $CD56^+CD16^+$ NK cells may reflect a reduced capacity for these cells to mediate antibody-dependent cellular cytotoxicity, a factor which could be significant to the disease process.

Finally, the percentage of $CD56^+$ NK cells co-expressing CD2, CD4 or CD8 did not differ significantly between PFS patients and normal controls, suggesting that these subsets of NK cells are not significantly involved in the immunopathology of this syndrome.

We found several changes in the phenotype of NK cells in patients with PFS, thus providing laboratory evidence of immunological abnormalities in this syndrome. These changes include significantly increased percentages of $CD56^+$, and especially $CD56^{bright+}$ NK cells, and $CD56^+CD25^+$, $CD56^+CD71^+$ and $CD56^+CD3^+$ subsets of NK cells. In contrast, the $CD56^+CD16^+$ subset of NK cells was significantly reduced in PFS patients. We suggest that these changes may be consistent with persistent viral infection in these patients.

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REFERENCES

ARCHARD, L.C., BOWLES, N.E., BEHAN, P.O., BELL, E.J. & DOYLE, D. (1988) Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatinine kinase. *J. R. Soc. Med.* **81,** 326.

ARNOLD, D.L., RADDA, G.K., BORE, P.J., STYLES, P. & TAYLOR, D.J. (1984) Excessive intracellular acidosis of skeletal muscle on exercise in a patient with postviral exhaustion/fatigue syndrome. *Lancet*, **i,** 1367.

BEHAN, P.O. & BEHAN, W.M.H. (1988) Postviral fatigue syndrome. *CRC Crit. Rev. Neurobiol.* **4,** 157.

BEHAN, P.O., BEHAN, W.M.H. & BELL, E.J. (1985) The postviral fatigue syndrome—an analysis of the findings in 50 cases. *J. Infect.* **10,** 211.

BUKOWSKI, J.F., BIRON, C.A. & WELSH, R.M. (1983) Natural cell-mediated cytotoxicity, plasma interferon and tumor cell rejection in mice persistently infected with lymphocyte choriomeningitis virus. *J. Immunol.* **131,** 991.

CALIGIURI, M., MURRAY, C., BUCHWALD, D., LEVINE, H., CHENEY, P., PETERSON, D., KOMAROFF, A.L. & RITZ, J. (1987) Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. *J. Immunol.* **139,** 3306.

CALIGIURI, M.A., ZMUIDZINAS, A., MANLEY, T.J., LEVINE, H., SMITH, K.A. & RITZ, J. (1990) Functional consequences of interleukin-2 receptor expression on resting human lymphocytes. *J. exp. Med.* **171,** 1509.

ENNIS, F.A., BEARE, A.S., RILEY, D., SCHILD, G.C., MEAGER, A., QUI, Y.H., SCHWARZ, G. & ROOK, A.H. (1981) Interferon induction and increased natural killer cell activity in influenza infections in man. *Lancet*, **ii,** 891.

GRIFFIN, J.D., HERCEND, T., BEVERIDGE, R.P. & SCHLOSSMAN, S.F. (1983) Characterization of an antigen expressed by human natural killer cells. *J. Immunol.* **130,** 2947.

HAMILTON, T., WADA, H.G. & SUSSMAN, H.H. (1979) Identification of transferrin receptors on the surface of human cultured cells. *Proc. natl Acad. Sci. USA*, **76,** 6406.

HERCEND, T., GRIFFIN J.D., BENSUSSAN, A., SCHMIDT, R.E., EDSON, M.A., BRENNAN, A., MURRAY, C., DALEY, J.F., SCHLOSSMAN, S.F. & RITZ, J. (1985) Generation of monoclonal antibodies to a human NK clone, characterization of two NK associated antigens NKH 1A and NKH 2 expressed on a subset of large granular lymphocytes. *J. clin. Invest.* **75,** 932.

JAMAL, G.A. & HANSEN, S. (1989) Postviral fatigue syndrome: evidence for underlying organic disturbance in the muscle fibre. *Eur. Neurol.* **29,** 272.

LANIER, L.L., LE, A.M., CIVIN, C.I., LOKEN, M.R. & PHILLIPS, J.H. (1986); The relationship of CD16 (Leu 11) and Leu 19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J. Immunol.* **136,** 4480.

LANIER, L.L., LE, A.M., PHILLIPS, J.H., WARNER, N.L. & BABLOCK, G.F. (1983) Subpopulations of human natural killer cells defined by expression of the Leu-7 (NKH-1) and Leu-11 (NK-15) antigens. *J. Immunol.* **131,** 1789.

LARRICK, J.W. CRESSWELL, P. (1979) Modulation of cell surface iron transferrin receptors by cellular density and state of activation. *J. Supramol. Struct.* **11,** 579.

MILLER, N.A., CARMICHAEL, H.A., CALDER, B.D., BEHAN, P.O., BELL, E.J., MCCARTNEY, R.A. & HALL, F.C. (1991) Coxsackie B serology does not help diagnose postviral fatigue syndrome. *Br. med. J.* (In press).

PERRIN, L.H., TISHON, A. & OLDSTONE, M.B.A. (1977) Immunological injury in measles virus infection. III. Presence and characterization of human cytotoxic lymphocytes. *J. Immunol.* **118,** 282.

PERUSSIA, B., TRINCHIERI, G., JACKSON, A., WARNER, N.L., FAUST, J., RUMPOLD, H., KRAFT, D. & LANIER, L.L. (1984) The Fc receptor for IgG on human natural killer cells: phenotypic, functional and comparative studies with monoclonal antibodies. *J. Immunol.* **133,** 180.

SHINDELMAN, J.E., ORTMEYER, A.E. & SUSSMAN, H.H. (1981) Demonstration of the transferrin receptor in human breast cancer tissue. Potential marker for identifying dividing cells. *Int. J. Cancer*, **27,** 329.

STRAUS, S.E., TOSATO, G., ARMSTRONG, G., LAWLEY, T., PREBLE, O.T., HENLE, W., DAVEY, R., PEARSON, G., EPSTEIN, J., BRUS, I. & BLAESE, R.M. (1985) Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. *Ann. intern. Med.* **102,** 7.

WELSH, R.M. (1981) Natural cell mediated immunity during virus infection. In *Natural Resistance to Tumours and Viruses* (ed. by O. Haller) p. 83. Springer-Verlag, Berlin.

YOUSEF, G.E., MANN, G.F., SMITH, D.G., BELL, E.J., MURUGESAN, V. & MCCARTNEY, R.A. (1988) Chronic enterovirus infection in patients with postviral fatigue syndrome. *Lancet*, **i,** 146.